MORPHOMETRIC, PHYSIOLOGICAL, HISTOLOGICAL, AND BIOCHEMICAL CHANGES IN RAT FOOT EXTENSORS IMMOBILIZED BY PLASTER

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Part I

Study of the Manifestation and Development of Muscular and Osseous Atrophy of the Immobilized Foot of the Rat

Introduction

Muscular atrophy, which consists in a decrease in volume and, /325** consequently, in weight, unveils to the investigators a large number of modifications of physiological and biochemical nature.

The myogenic or primary causes belong to the category of myopathies. The secondary causes [39] can either be neurogenous — i.e., due to organic unbalance because of undernutrition or organic malnutrition, circulatory problems or aging — or reflex, i.e., they

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**Numbers in the margin indicate pagination in the original foreign text.

may be the result of infection, inflammation, pain, trauma [38], or immobilization [25 - 33, 35].

Muscular atrophy [28] can be caused by toxic substances and chemicals; muscular undernutrition caused either by malnutrition or decreased circulation can give the same symptoms. The study of muscular denervation, thoroughly investigated by Gutman [15] and Garcia-Bunel [12], has made possible the publication of several interesting works on the study of the muscles [14, 15, 16].

Chor and Dolkart [6] immobilized the leg of a monkey in a cast and observed the progressive evolution of atrophy. The studies carried on by Eccles [9] clearly indicate that atrophy caused by tenotomy is more pronounced that that produced by immobilization.

Fischlach [11], following the example of Solandt [31], used a stainless steel apparatus to immobilize the leg and obtained muscular atrophy without any pathological lesion.

Deitrick [7] and Reid [30], in their investigation with normal individuals, obtained the same results.

Japanese workers have done some very interesting studies on muscular atrophy. Ikai [18] analyzes the various factors which influence muscular strength; Kato [20] studies muscular atrophy by immobilizing the limb with adhesive bandage; Esaki [10], using the same technique of immobilization, shows that the fasciculi of atrophied muscles lose their properties. Kurakami [21], using the same method, studies the modifications occurring in the various muscular components. Finally, Takaki [36] studies mitochondria and observes a decrease in diameter and a dilatation of the cavities of the T system.

It is interesting to study muscular atrophy using a cast, since this is the most common cause of immobilization in man, and to observe if atrophy begins very soon and then progresses with time.

Materials and Methods

The experiments were carried out using albino rats of the Wistar strain of unknown origin, raised at the nutrition laboratory of the Catholic University of Louvain. The rats, free of outside contamination for a period of 25 years, were six months of age and weighed approximately 300 grams.

They were kept in cubic cages with 40 cm sides. There was one rat per cage. Feces were eliminated through a mesh at the bottom of each cage.

Food was administered through a 50 cm long plastic tube attached at the ceiling of the cage. The pellets filling the tube were held at the lower end by a U-shaped plug. By this method, it was possible to measure exactly the amount of food used each day by each animal. Food and water were ad libitum throughout the experimental period.

Method of immobilization: Rats were anesthetized using cotton saturated with ethyl ether, and, if they should happen to come out of anesthesia during the immobilization procedures, ether was again administered in the same way.

Plaster was used to immobilize the foot and the plaster cast, 5 cm in width, was cut in pieces of 12 cm in length. Eight to ten pieces were used to immobilize the foot.

Before applying the plaster, the limb was covered with light plastic to facilitate the removal of the plaster, and to avoid its adhering to the skin.

Both leg and foot were covered with plaster. The foot was put /326
in a 90 - 95° dorsal flexion. On the outside, the plaster was shaped so as to allow the rat to move as fast as possible. By smoothing the upper part of the cast at the knee level, it was possible to avoid inflammation and wounds.

At the end of the immobilization period, in order to free the leg from the plaster without bruises, the leg in the cast was soaked in water at 37° C. Then the back portion was cut with a saw and the leg was removed using a tweezer.

Method of dissecting the extensors: This dissection method allows the investigator working alone to remove the soleus from the foot, and to put it on the ergometer within 3 to 4 minutes.

The foot of the rat slightly bent is suspended from a horizontal rod placed above the animal. Starting from the lower part of the external malleolus, the skin is removed with a scalpel. A string attached to the Achilles tendon, makes it possible to dissect this part while having a holding point to free the soleus from the remaining extensors.

Assays: The assays for the calcium of the bone and the nitrogen of the muscle were carried on according to the classical methods [23]. At the present time, the Conaway method [5] is preferred for the determination of the levels of the mineral salts. The histological samples were prepared according to the technique of Langeron [22] and Bouin [2], except for the fact that we suspended the muscle by a tendon in the "Bouin fluid" and that a 2-g glass weight attached to the other tendon was completely inhibiting any muscular contraction.

Planning the experiments:

A total of 17 rats were used for the experiment. They were divided into two groups, one of five and the other of 12 animals. The first group (A) was immobilized during a period of 3, 6, 10, and 17 days. The second group (B) was immobilized during a period of 4, 8, 12, 16, 20, and 24 days. For each atrophied rat there was a control, in order to compare the results. /327

TABLE 1. WEIGHTS OF RATS AND WEIGHTS OF THE ATROPHIED MUSCLES AND OF THEIR CONTRALATERAL ONES. FIGURES GIVEN ARE EXPRESSED IN GRAMS

Days immobilized		0	3	6	10	17
Body wt. (g)		308	276	288	269	249
Extensors wt. (mg)	Immobilized (right)	1934	1586	1562	1493	1403
	Contralateral (left)	1908	1753	1921	1842	1775
Gemelli wt. (mg)	Immobilized (right)	1493	1219	1208	1192	1106
	Contralateral (left)	1477	1358	1492	1410	1393
Plantaris wt. (mg)	Immobilized (right)	320	265	263	215	227
	Contralateral (left)	310	270	305	293	275
Solei wt. (mg)	Immobilized (left)	121	102	91	86	70
	Contralateral (left)	121	125	124	139	107

TABLE 2. PERCENTAGE OF ATROPHY OF THE EXTENSORS OF THE RIGHT FOOT IN THE EXPERIMENTAL ANIMALS AS COMPARED WITH THE EXTENSORS OF THE LEFT FOOT (COLUMN A) AND WITH THE MUSCLES OF THE CONTROL FOOT (COLUMN B)

Days	Extensors		Solei		Plantaris		Gemelli	
	A	B	A	B	A	B	A	B
3	10	18	19	16	2	16	11	18
6	19	19	27	25	14	17	20	19
10	19	23	39	29	27	32	16	20
17	25	23	35	43	16	28	21	26

Results

A. The results obtained with animals of group A show that very little difference exists between the weights of the right and left normal muscles. Table 1 gives the results relating to the weights of the muscles which had been immobilized as compared to their contralateral ones.

The percentage in weight loss of the muscles which had undergone immobilization is given in Table 2.

TABLE 3. WEIGHTS OF ATROPHIED MUSCLES, CONTRALATERALS AND CONTROLS

			No. of days of immobilization					
			4	8	12	16	20	24
Solei (mg)	Atrophied muscles	Right	106	101	97	90	83	74
		Left	135	152	121	138	145	133
	Controls	Right	112	140	129	115	114	101
		Left	120	142	129	116	117	101
		Average	116	141	129	115	115	101
Plantaris (mg)	Atrophied muscles	Right	283	252	292	232	206	179
		Left	314	329	305	324	295	292
	Controls	Right	307	322	305	303	282	281
		Left	309	328	321	306	313	270
		Average	308	325	313	304	298	276
Gemelli (mg)	Atrophied muscles	Right	1291	1152	1138	1018	912	840
		Left	1405	1495	1650	1494	1527	1522
	Controls	Right	1515	1542	1525	1443	1444	1409
		Left	1576	1556	1490	1363	1396	1429
		Average	1545	1549	1508	1403	1420	1419
Extensors (mg)	Atrophied muscles	Right	1680	1505	1527	1340	1201	1093
		Left	1854	1976	2076	1956	1967	1947
	Controls	Right	1934	2004	1959	1861	1840	1791
		Left	2005	2026	1940	1785	1826	1800
		Average	1969	2015	1950	1822	1833	1796

The ratio between the total weights of the atrophied extensors and the number of days of immobilization is 0.83.

Figure 1 shows the evolution of the atrophy curve during a period of immobilization from 3 to 17 days.

B. From the results of group B, it appears that the growth of the animals in the cast has been slowed down in comparison to that of the controls. This phenomenon is

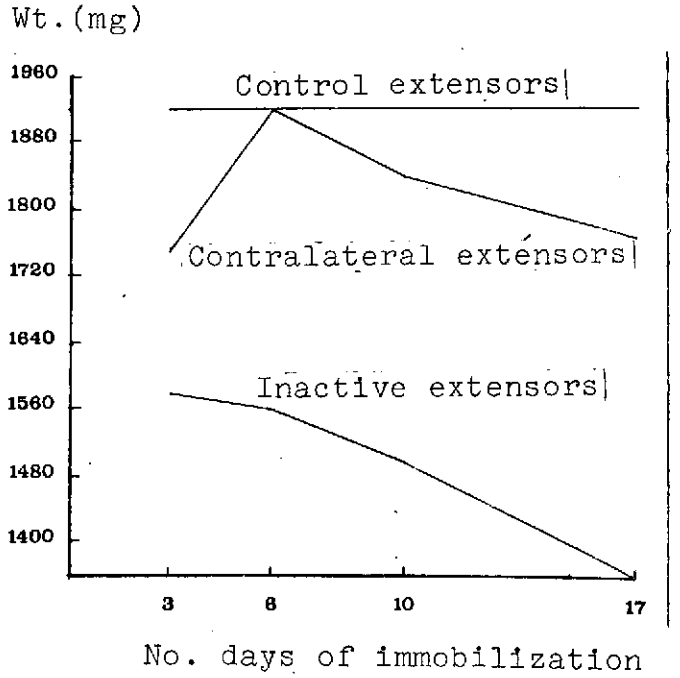

Figure 1. Evolution of muscular atrophy of extensors as compared to contralateral muscles and to normal ones

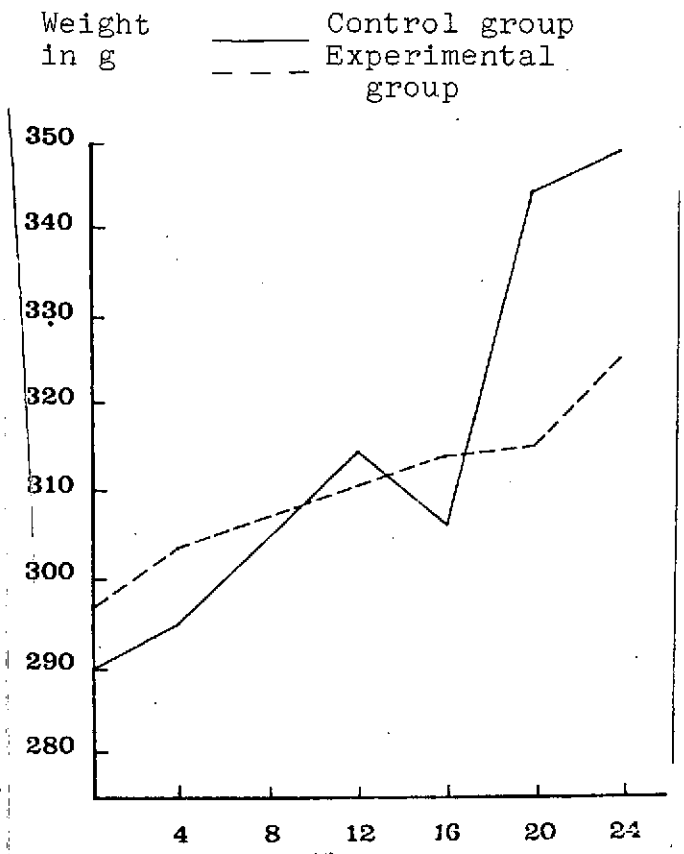

Figure 2. Evaluation of weights of experimental and of control animals

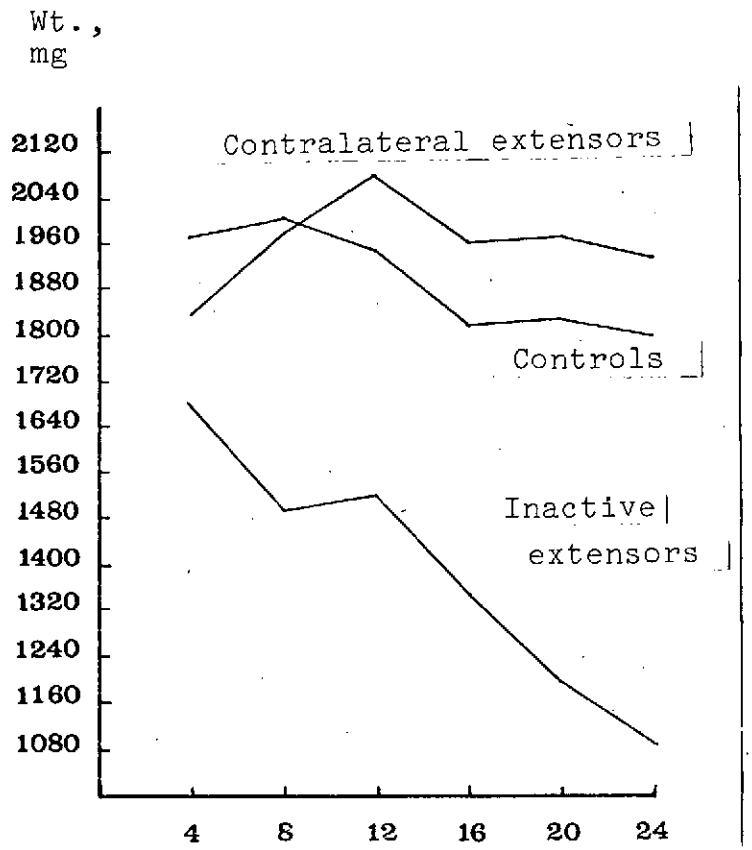

Figure 3. Evolution of muscular atrophy of the extensors as compared to the contralateral muscles and to normal ones

particularly evident during the first days of the experiment. In Figure 2, the development of the weight of the animals is given as a function of the number of days.

In Table 3, the weight of the muscles has been calculated by referring to a unitary body weight in order to eliminate one of the sources of variations from the experimental error.

The effect of immobilization can be better demonstrated by expressing the loss in muscular weight as a percentage of the control values (Table 4). /328

TABLE 4. PERCENTAGE OF ATROPHY OF THE EXTENSORS OF THE RIGHT FOOT AS COMPARED TO THE OTHER FOOT (A) AND TO THE AVERAGE VALUE OF THE CONTROL MUSCLES (B)

Days immobil.	Extensors		Solei		Plantaris		Gemelli	
	A	B	A	B	A	B	A	B
4	10	15	22	9	10	9	9	17
8	24	26	34	29	24	23	25	26
12	27	22	20	25	5	7	32	25
16	32	27	35	22	29	24	32	28
20	39	35	43	28	31	31	41	36
24	44	40	45	27	39	36	45	49

The weight curve of all the extensors of the back limbs of 12 rats indicates an atrophization process progressing with the number of days (Figure 3).

The ratio between the total weights of the atrophied extensors and the number of days of immobilization is 0.98. The curve of Figure 4 shows that atrophy increases with the number of days of immobilization.

The change of the osseous weights and of the calcium content of each tibia and each perone of all hind feet is indicated in Table 5. /329

Figure 5 shows the variation of the osseus weight loss and the decrease and increase in calcium content as a function of the immobilization duration in days. The correlation in calcium content and the immobilization duration increases to 0.83. Figures 6 and 7 show the radiographs of these bones.

Figures 8 and 9 show the histological sections corresponding to a soleus immobilized for 24 days and its mate from the other members. The enlargement factor is the same for both figures.

TABLE 5. VALUES OBTAINED DURING THE VARIOUS STAGES OF MEASUREMENT OF TIBIAE AND PERONI OF EXPERIMENTAL AND CONTROL RATS

Rat no.	Rat weight (g)	Limb	Bone weight (fresh) (mg)	Bone weight (dry) (mg)	Weight of minerals (mg)	Amount calcium (mg)	% Ca/ mineral content of bone
Exp. 1	281	Right	867	460	240	44	18
		Left	912	481	248	46	18
Control 1	305	Right	1108	494	278	48	17
		Left	1114	490	270	47	17
Exp. 2	290	Right	698	393	224	42	18
		Left	721	435	237	48	20
Control 2	302	Right	968	456	240	46	19
		Left	875	431	239	45	18
Exp. 3	323	Right	830	499	280	55	19
		Left	944	517	298	67	22
Control 3	354	Right	1065	490	279	48	17
		Left	1110	550	294	50	17
Exp. 4	326	Right	1120	550	287	52	18
		Left	1080	559	322	60	18
Control 4	239	Right	871	419	247	45	18
		Left	863	419	256	46	17
Exp. 5	303	Right	851	470	240	42	17
		Left	985	475	260	49	18
Control 5	342	Right	1058	500	257	51	19
		Left	992	502	262	52	19
Exp. 6	324	Right	903	470	230	40	17
		Left	970	501	285	58	20
Control 6	353	Right	1100	500	270	55	20
		Left	1050	470	261	54	20

Discussion

The interest and originality of our study depends on the fact /330
that atrophy follows a progressive pattern of development in time, that the study of the atrophy process starts the third day of immobilization, that each experimental rat has its control, and that it /331
is possible to establish a relationship between muscular and osseous loss.

Chor and Dolkart [6] have found in monkeys a muscular atrophy of 12.8% after 15 days, and of 29.8% after 50 days. Our study, on

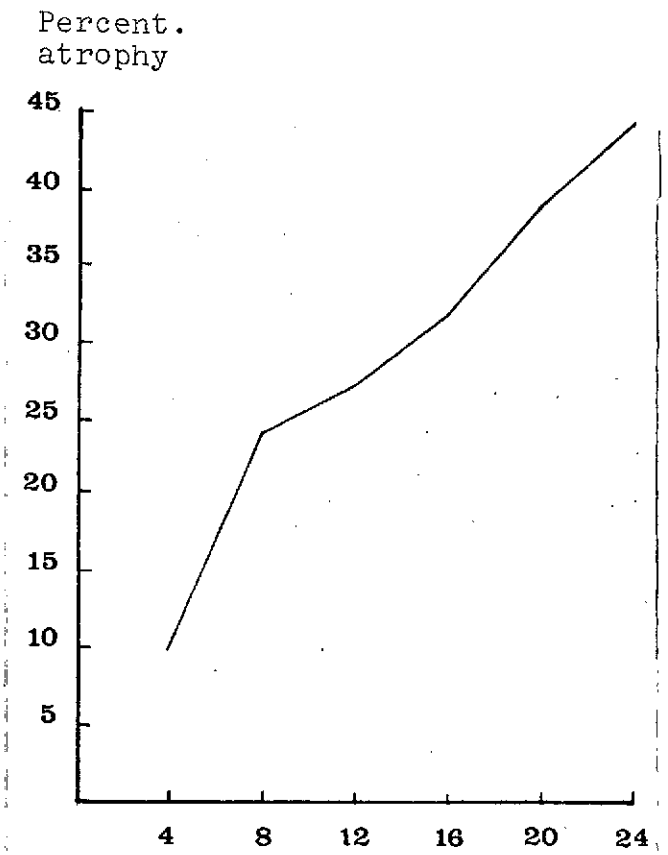

Figure 4. Atrophy as a function of the number of days of immobilization. The curve shows the percentage of atrophy of extensors of the immobilized limb, as compared to the contralateral muscles

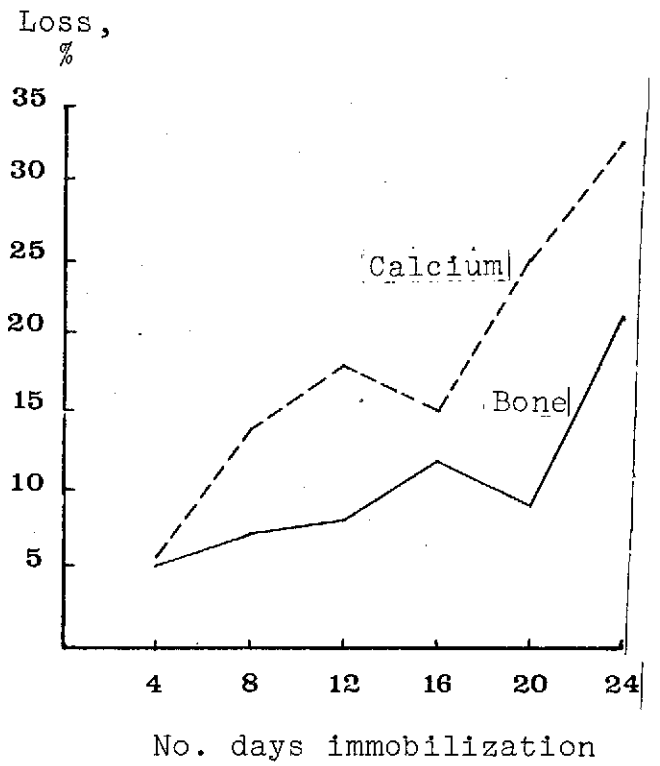

Figure 5. Progressive loss in weight and in calcium level of the bone

the contrary, shows a muscular atrophy of 10% after 4 days, of 32% after 16 days, and of 44% after 24 days, for the four extensors of the hind foot of the rat. These differences can be explained on the basis of the fact that monkeys use their hind limbs to move. This requires a much greater amount of inert work during immobilization, while rats move using their four feet and leave the cast at the bottom of the cage.

Following immobilization, the extensor muscles, whether of the red or of the white type, undergo approximately the same type of change.

The progressive weight loss of the bone and its calcium level follow the curve of the immobilization period. It is interesting to

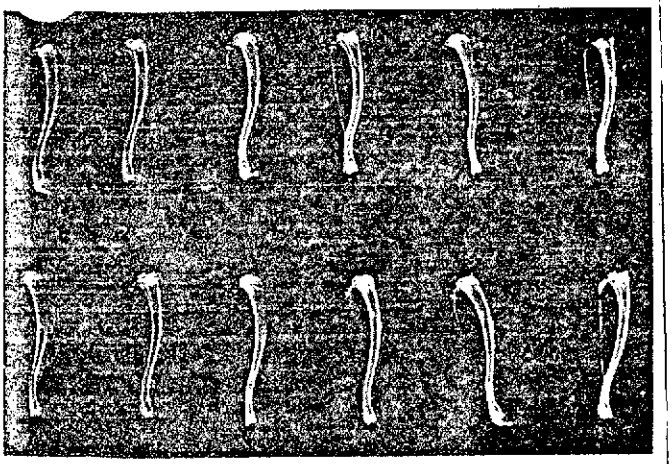

Figure 6. Radiography of tibiae and peroni. From left to right and from top to bottom:

1 — bone immobilized for 4 days; 2 — contralateral; 3, 4 — bone of control rat; 5 — bone immobilized for 8 days; 6 — contralateral; 7, 8 — bone of control rat; 9 — bone immobilized for 12 days; 10 — contralateral; 11, 12 — bone of control rat

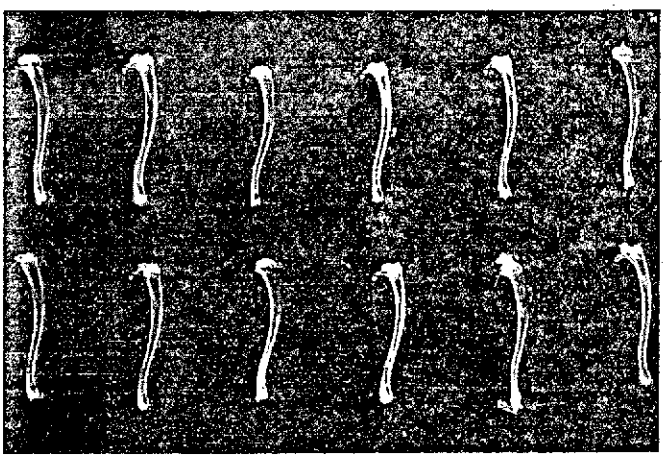

Figure 7. Radiography of tibiae and peroni. From left to right and from top to bottom:

1 — bone immobilized for 16 days; 2 — contralateral; 3, 4 — bone of control rat; 5 — bone immobilized for 20 days; 6 — contralateral; 7, 8 — bone of control rat; 9 — bone immobilized for 24 days; 10 — contralateral; 11, 12 — bone of control rat

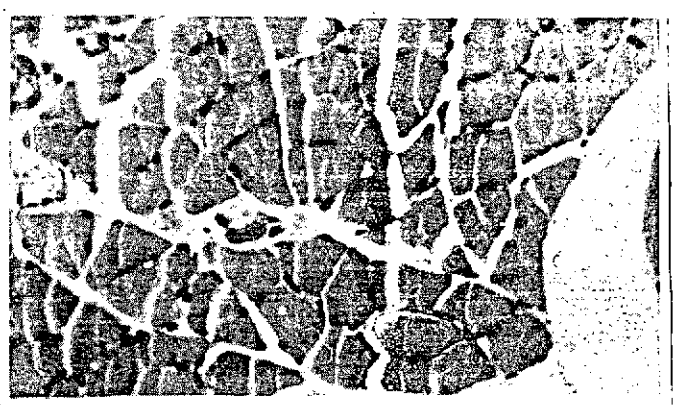

Figure 8. Transverse section of the central portion of the soleus immobilized for 24 days

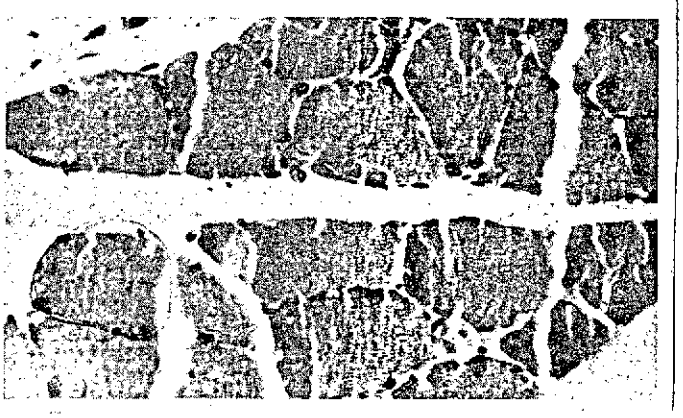

Figure 9. Transverse section of the central portion of the soleus in the contralateral of the limb immobilized for 24 days

observe that the calcium level in the bone remains constant in comparison with the total weight of the bone itself, and that is 19% of the total weight.

As appears from histological studies, the volume of the muscle fibers decreases at the same rate at the center and at the edges.

On the basis of these observations, we found that following immobilization muscular loss follows the same pattern as osseous loss.

Summary of Part I

The author undertook investigations in order to find out whether muscular atrophy due to immobilization, without anatomical modification, begins from the first days and develops progressively with time.

The data from the volumetric analysis of the muscles and from the determination of calcium level in the bones indicate that atrophy due to immobilization begins rapidly and progresses with time. The percentage of muscular atrophy corresponds to the percentage of calcium loss.

Part II
Study of Some of the Morphometric, Physiological, and Biochemical Parameters of the Extensors, Immobilized in Plaster

Introduction

The works of Summers [34], Ralston [29], and Stilwell [33] point out the influence of the position of the immobilized limb upon its atrophy and show the means to adopt in order to decrease it.

During the last five years, other investigators, mainly Dreyfus [8] and Goldberg [13], have pointed out a very pronounced proteinic

catabolism following denervation. Furthermore, Srivastava [32] and Jakubzak [19] have clearly shown that age causes a decrease in muscle weight due to proteinic catabolism. This latter kind of atrophy emphasizes more certain problems related to metabolism than problems due to immobilization.

The results of these investigations prompt us to conclude that atrophy due to immobilization of a limb without any anatomical modification causes in the limb a relatively constant loss of its building elements and a decrease of its muscular tension, without causing a slow-down of its speed of contraction. In the first part of our work, we give the necessary information relating to the animals and describe the techniques adopted to induce atrophy and to dissect the extensor muscles.

In this second part, we shall study the influence of a 24-day period of immobilization upon the extensors of the hind foot of the rat. The various morphometric, physiological, and biochemical parameters will be systematically analyzed and compared with those of the control groups.

Materials and Methods

The instruments and the methods of analysis, pneumatic ergometer and tensiometer, used are described in Maréchal [27].

A metallic rod with two pairs of three electrodes, a pair located at each end of a small chain 30 cm long, make it possible to control the length of the muscle during the experiment. The end of the rod is immersed in a container with double wall where water at a desired temperature is circulated. The container, while allowing us to put the muscle inside the desired solution, has a special opening which allows the passage of a gas.

Above the rod there is a cylinder of a pneumatic ergometer together with the tensiometers, and close to it is the cylinder that regulates the speed with an oil valve to regulate the movement.

To the left of the equipment, a frame embodying all the transistorized systems makes it possible to direct the experiment.

Under this apparatus, we have placed an oscillograph and a recorder with double track. The paper used is the Sanborn recording paper.

An electric current of 220 volts feeds these devices. However, the current used to stimulate the muscle is furnished by a 100-volt direct current battery.

This type of instrument can be used for different studies. In our work it measures the tension of the muscle at different lengths and the speed-force using different speeds of contraction.

The soleus, attached by its two tendons, is placed between two lines of platinum electrodes fed by alternate discharges from a condenser and originating from a multi-vibrator connected with a dc battery.

The muscle is immersed in a Krebs solution containing glucose (Table 6) and kept at a constant temperature of 30° C. by a circulating water bath. A mixture of 95% oxygen and 5% carbon dioxide is injected into this solution.

Every three minutes, the soleus is stimulated with a current of /332 one volt, having a frequency of 50 cycles per second, for 1.5 seconds. The first stimulus is applied to the muscle having a standard length (in situ length) of between 35 and 36 mm. Then 2 mm of length are added for each one of the two other stimuli. Figure 10 shows the shapes of the three curves. The curve chosen is the one

TABLE 6. COMPONENTS OF THE KREBS SOLUTION WITH GLUCOSE

Compounds	Amount, mg
NaCl	6.9
KCl	0.35
$CaCl_2.2H_2O$	0.37
$MgSO_4.7H_2O$	0.29
$NaHCO_3$	2.10
KH_2PO_4	0.185
Glucose	1.00

indicating the highest tension. In this case, it is contraction B, exhibiting a tension of 165 g.

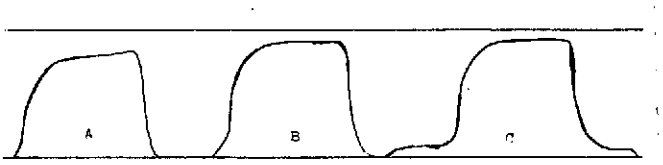

Figure 10. Three isometric contractions of the control soleus number three. A = length L0; B = Lo + 2 mm; C = Lo + 4 mm

The speed of contraction is found by setting up the muscle at the length where it develops the highest tension, i.e., at Lo or at Lo + 2 mm. Lo is defined as the reference (or standard) length of the muscle in situ.

Our solei undergo a 4 mm contraction by using a double mirror surface: 4 - 10 - 6 - 8 - 8 - 6 - 10 - 4. These figures correspond, in the same order, to speeds of contraction of 0.18; 1.43; 0.40, and 0.80 cm per second.

The soleus begins to contract 0.5 seconds after the stimulus [3].

A constant nitrogen pressure of 4.5 kg per cm^2 acts upon the oil. This oil, more or less viscous depending on the requirements, determines the speed of contraction by acting as a brake.

Aubert [1] has used the formula $Pr = Po\ e^{-V/B}$ in order to find the speed constant of the muscles he used. This formula can also be written as:

$$B = \frac{V}{Ln \times \frac{Po}{Pr}}$$

where B is the speed constant, V — the speed of contraction of the muscle, Po — the maximum value of tension, and Pr — the tension immediately following the beginning of the contraction. /333

Figure 11 gives the force-speed relationships of a muscle using two different lengths of contraction.

Experimental procedure. Twenty rats were divided into two groups: the experimental and the control group.

Statistical analysis shows that the rats belong to a normal population with an average weight of 300 g, and that our hypothesis of uniformity still holds true even by allowing for a large probability error (P = 0.25).

Results

Table 7 gives the weights of the animals, as well as the weight of the food and of the water. An amount of 7.78 mg of protein per g of weight per day is

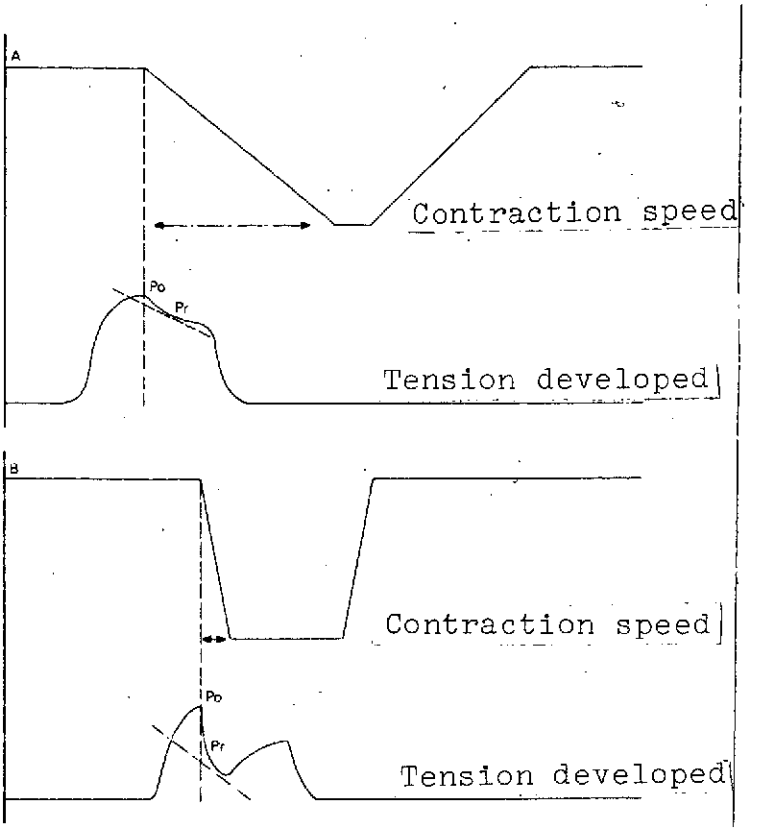

Figure 11. A — slow speed of contraction of the soleus (0.18 cm/sec); B — rapid speed of contraction of the soleus (1.43 cm/sec

TABLE 7. WEIGHT OF THE ANIMALS IN g, WEIGHT OF THE FOOD IN g, WEIGHT OF THE LIQUID CONSUMED IN g, AND NUMBER OF CALORIES*

Rat No.	Experimental rats				Control rats			
	Weight	Food/ day	Calories/ day	Water/ day	Weight	Food/ day	Calories/ day	Water/ day
1	314	16,5	53,95	22	337	15,5	50,68	28,5
2	272	13	42,51	16	383	21,5	70,30	35
3	300	16,5	53,95	20,5	342	14,5	47,41	29
4	289	13	42,51	20	261	12	39,24	21,5
5	303	19	62,13	22,5	300	16,5	53,95	26
6	325	18	58,86	29	282	16,5	53,95	23
7	291	13,5	44,14	23	307	22,5	73,57	26
8	259	14	45,78	21	300	17,5	57,22	23,5
9	267	12	39,24	21,5	275	14	45,78	23
10	292	14,5	47,41	22,5	302	19,5	63,76	29,5
$\bar{X}$	291	15	49,05	21,7	309	17	55,59	26,5

*Translator's note: Commas represent decimal points.

used by the experimental animals, as compared to 7.96 mg for the control rats.

Figure 12 illustrates the lines of regression indicating the change in weight of the animals.

Morphometric results. The atrophy of the foot extensors, whether tonus muscles, such as the soleus and the plantaris, or phase muscles, such as the gemelli, has been studied muscle by muscle.

Table 8 gives the data referring to the extensors.

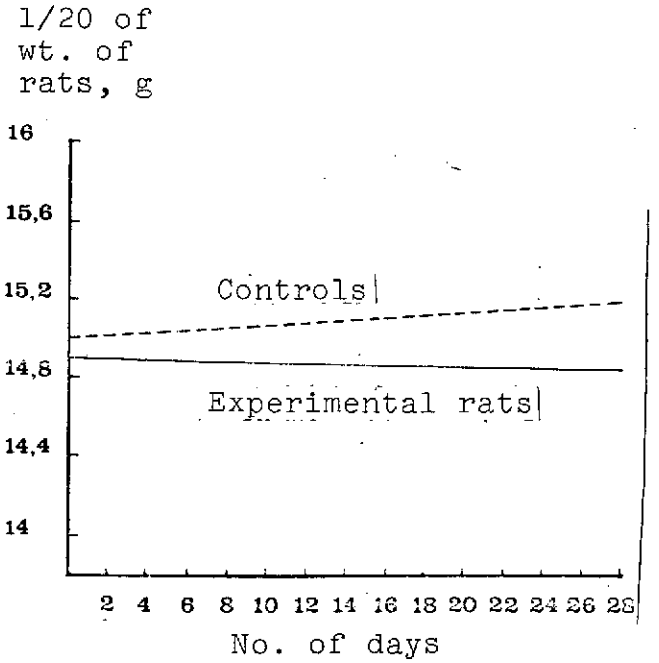

Figure 12. The two lines of regression. Changes in body weight of experimental rats and of control rats during immobilization

experimental rats: b = -0.029 a = 14.918; control rats: b = 0.035 a = 15.008

When the weight of the solei, plantaris and gemelli are compared to the weight of their contralaterals, an average atrophy value of 39.62% is obtained. The average weight of these muscles, when compared to the standard average of the right and left muscles of the control rats, shows an atrophy of 39.97%.

Table 9 gives the analysis of variance between the weight of the extensors of the immobilized foot and the weight of their contralaterals.

The results obtained show that plaster immobilization causes significant atrophy of the muscles.

Table 10 gives the analysis of covariance between the weight of the experimental rats and the weight of the control rats, the weight of the atrophied extensors, and the average weight of the extensors of the control rats. /334

TABLE 8. WEIGHT OF THE ANIMALS AND OF THE EXTENSORS IN g*

Group rat no.	Rat wt.	Weight of right muscles				Weight of left muscles			
		Soleus	Plantaris	Gemelli	Extensors	Soleus	Plantaris	Gemelli	Extensors
1	319	0,125	0,241	1,118	1,484	0,206	0,291	1,412	1,909
2	261	0,085	0,139	0,812	1,036	0,207	0,257	1,465	1,929
3	292	0,090	0,169	0,781	1,040	0,159	0,273	1,530	1,962
4	291	0,124	0,181	0,689	0,994	0,180	0,308	1,482	1,970
5	303	0,103	0,240	1,032	1,425	0,170	0,341	1,607	2,118
6	334	0,156	0,280	1,363	1,799	0,229	0,337	1,570	2,136
7	276	0,144	0,231	0,906	1,281	0,195	0,293	1,350	1,838
8	277	0,112	0,178	0,602	0,892	0,184	0,241	1,244	1,669
9	252	0,116	0,141	0,475	0,732	0,163	0,271	1,230	1,664
10	277	0,125	0,180	0,739	1,044	0,244	0,327	1,509	2,080
$\bar{X}$	288,2	0,118	0,198	0,857	1,173	0,194	0,294	1,440	1,927
1	340	0,206	0,342	1,635	2,183	0,258	0,337	1,649	2,244
2	414	0,220	0,355	1,756	2,331	0,208	0,393	1,751	2,352
3	357	0,172	0,345	1,665	2,182	0,184	0,355	1,753	2,292
4	254	0,132	0,242	1,160	1,534	0,124	0,233	1,241	1,598
5	304	0,173	0,307	1,416	1,896	0,184	0,316	1,425	1,925
6	286	0,151	0,298	1,474	1,923	0,147	0,298	1,424	1,869
7	314	0,196	0,352	1,608	2,156	0,207	0,350	1,595	2,152
8	305	0,225	0,329	1,795	2,349	0,208	0,337	1,653	2,198
9	278	0,191	0,282	1,402	1,875	0,191	0,294	1,377	1,862
10	315	0,204	0,304	1,409	1,917	0,227	0,292	1,387	1,906
$\bar{X}$	316,7	0,187	0,316	1,532	2,035	0,194	0,320	1,525	2,040

TABLE 9. ANALYSIS OF VARIANCE BETWEEN WEIGHT OF EXTENSORS OF THE FOOT IMMOBILIZED AND WEIGHT OF THEIR CONTRALATERALS*

Source of variation	Sum of squares	DL	Mean value of squares	F
Between groups	1731072,8	1	1731072,8	4,621*
Error	7310801,	18	406155,6	
Totals	9041873,8	19		P = 0,05

* Translator's note: Commas represent decimal points.

TABLE 10. ANALYSIS OF COVARIANCE BETWEEN WEIGHT OF THE EXPERIMENTAL ANIMALS AND WEIGHT OF CONTROL RATS, WEIGHT OF THE ATROPHIED EXTENSORS AND MEAN WEIGHT OF THE EXTENSORS OF THE CONTROL RATS*

Source of variation	DL	Sx^2	Sxy	Sy^2	$\frac{(Sxy)^2}{Sx^2}$	Sy^2 red	DL	Mean square
Treatment	1	4061,25	123,081	3,7416		2,0757	1	2,0757
Error	18	24051,7	142,6717	1,4288	0,8463	0,5825	17	0,0343
Total	19	28112,95	265,7527	5,1704	2,5122	2,6582	—	60,52***

TABLE 11. CORRECTED AVERAGE WEIGHT OF ATROPHIED EXTENSORS AND OF CONTROL EXTENSORS, AND PERCENTAGE OF ATROPHY. X_a = WEIGHT OF CONTROL RATS; Y_a = WEIGHT OF THE ATROPHIED EXTENSORS; Y_b = AVERAGE WEIGHT OF THE EXTENSORS OF THE CONTROL RATS*

$b = \frac{Sxy_e}{Sx_e^2}$	$\bar{Y}_a = \bar{Y}_a + b(\bar{X} - \bar{X}_a)$	$\bar{Y}_b = \bar{Y}_b + b(\bar{X} - \bar{X}_b)$	Percentage atrophy
= 0,0059	= 1,1611	= 2,0386	42,06

* Translator's note: Commas represent decimal points.

The difference of correlation between the weight of the control rats and the average weight of their extensors is closer to one (R = 0.82) than the difference between the weight of the experimental rats and that of their contralateral extensors (R = 0.63).

Table 11 gives the final formulas for the correction of the average weights of the atrophied extensors and for the correction of the extensors of the control rats, as well as the percentage of atrophy.

This actual atrophy of 42.06% is higher than the preceding ones, and it can be explained on the basis of the statistical analysis where the adjustment for all the weights is being taken into account [26].

Variation of Nitrogen Concentration in Normal Muscles and in Atrophied Muscles

The results of these measurements are given in Table 12. The average percentage of loss in protein is 33.57% as compared to the /335
contralateral muscles, and 37.2% as compared to the average value for the solei of the control rats. This last figure is multiplied by a correction factor in order to make a comparison on the basis of a common figure which is the weight of the animals. The average percentage of the muscle protein in relation to the weight remains rather constant. The value is 15.76% for the atrophied muscles, and 14.59% for their contralaterals and 16.58% for the controls.

The analysis of variance (Table 13) between the atrophied muscles /336
and their contralaterals is again highly significant.

Physiological Study of the Atrophied Muscle and of the Control Muscle

Table 15 gives the maximum tension values attained by the experimental muscles and by the control muscles. This tension is found when the muscle has its *in situ* length "lo" plus 2 mm.

The solei of our experimental rats develop a maximum tension of 86.6 g, as compared to 164.6 g which is the value observed in the control animals. The difference between experimental animals and controls is, therefore, 47.38%. The difference of 46.65% given in Table 14 is due to the difference in tension measured individually for each muscle.

The analysis of variance (Table 15), which is highly significant, between the tension of the experimental solei and the tension of the control ones, confirms the hypothesis that the fall in tension of the soleus is proportional to its muscular loss.

TABLE 12. DATA RELATING TO THE NITROGEN CONTENT OF THE MUSCLES. THE PROTEIN LEVEL IS FOUND IN RELATION TO THE WEIGHT OF THE SOLEARIS AND ITS PERCENTAGE ATROPHY,*

Group	Rat no.	Rat weight (g)	Daily consumption protein (g)	Wt. of solearis (mg)		Amt. of nitrogen (mg)		Amt. of protein (mg)		Percent. protein in muscle		% loss
				D	G	D	G	D	G	D	G	
Experimental	1	319	2,44	125	206	2,91	5,02	18,19	31,37	14,55	15,23	42,01
	2	261	1,92	85	207	2,20	4,68	13,75	29,25	16,18	14,13	52,99
	3	292	2,44	90	159	2,33	3,94	14,56	24,62	16,18	15,49	40,86
	4	291	1,92	124	180	3,11	4,75	19,44	29,69	15,68	16,59	34,52
	5	303	2,81	103	170	3,02	4,84	18,87	30,25	18,33	17,79	37,62
	6	334	2,66	156	229	3,70	4,11	23,12	25,69	14,82	11,22	10,00
	7	276	2,00	144	195	3,55	4,20	22,19	26,25	15,41	13,46	15,47
	8	277	2,07	112	184	2,53	3,84	15,81	24,00	14,12	13,04	34,13
	9	252	1,78	116	163	2,80	3,89	17,50	24,31	15,09	14,92	28,01
	10	277	2,15	120	244	3,30	5,51	20,62	34,14	17,19	14,11	40,13
	X̄	288,2	2,22	117,5	193,7	2,94	4,48	18,40	27,99	15,76	14,59	33,57
Control	1	340	2,29	206	258	5,64	5,89	35,25	36,81	17,11	14,27	
	2	414	3,18	220	208	5,50	5,12	34,37	32,00	15,63	15,38	
	3	357	2,15	172	184	4,17	5,48	26,06	34,25	15,15	18,61	
	4	254	1,78	132	124	4,03	3,97	25,19	24,81	19,08	20,01	
	5	304	2,44	173	184	5,28	5,80	33,00	36,25	19,08	19,70	
	6	286	2,44	151	147	4,53	3,94	28,31	24,62	18,75	16,75	
	7	314	3,33	196	207	4,85	5,30	30,31	33,12	15,47	16,00	
	8	305	2,59	225	208	6,04	5,55	37,75	34,69	16,78	16,68	
	9	278	2,07	191	191	5,43	5,40	33,94	33,94	17,77	17,77	
	10	315	2,89	204	227	5,18	5,82	32,37	36,37	15,87	16,02	
	X̄	316,7	2,52	187	193,8	5,06	5,23	31,65	32,69	17,07	16,02	

*Translator's note: commas represent decimal points.

In the relationship between force and speed, the different speed constants of the solei muscles of the experimental rats and of the control ones are summarized in Table 16.

Each speed constant is the average obtained with eight data for each value. Figure 13 gives the lines of regression for the experimental and the control solei. The estimated speed is plotted on the Y axis and the Ln Po/Pr is plotted on the X axis. Our lines are assembled from either the experimental or the control group.

TABLE 13. ANALYSIS OF VARIANCE OF THE VARIATION BETWEEN THE AMOUNT OF PROTEINS IN THE IMMOBILIZED SOLEI AND THE ONE ENCOUNTERED IN THEIR CONTRA-LATERALS*

Source of variation	Sum of squares	DL	Mean square	F
Between groups	459,0736	1	459,0736	42,6178***
Error	198,5525	18	11,0307	
Totals	657,6261	19		P = 0,001

TABLE 14. TENSIONS DEVELOPED BY THE ATROPHIED SOLEARIS AND BY THE RIGHT SOLEARIS OF THE CONTROL RAT ARE EXPRESSED IN GRAMS*

Rat no.	Experimental			Control			% atrophy
	Rat wt.	Muscle wt.	Muscle tension	Rat wt.	Muscle wt.	Muscle tension	
1	319	125	82	340	206	156	47,44
2	261	85	55	414	220	146	62,33
3	292	90	79	357	172	165	52,12
4	291	124	100	254	132	160	37,50
5	303	103	110	304	173	180	38,89
6	334	156	99	286	151	130	23,85
7	276	144	102	314	196	150	32,16
8	247	112	70	305	225	185	62,16
9	252	116	69	278	191	182	62,09
10	277	125	100	315	204	192	47,92
$\bar{X}$	285,2	118	86,6	316,7	187	164,6	46,65

TABLE 15. THIS ANALYSIS OF VARIANCE SHOWS THE VARIATION BETWEEN THE TENSIONS DEVELOPED BY THE IMMOBILIZED SOLEI AND BY THE CONTROL SOLEI*

Source of variation	Sum of squares	DL	Mean square	F
Between groups	30420,	1	30420	84,0***
Error	6518,8	18	362,1516	
Totals	36938,8	19		P = 0,001

*Translator's note: commas represent decimal points.

TABLE 16. SPEED CONSTANTS OF THE RIGHT SOLEI MUSCLES OF THE EXPERIMENTAL RATS AND OF THE CONTROL RATS IN cm/sec*

Group		
Rat no.	Experimental	Control
1	1,52	1,56
2	2,0	2,15
3	2,46	2,02
4	1,9	1,64
5	1,51	1,85
6	1,59	1,75
7	1,84	1,77
8	1,55	1,58
9	1,78	1,53
10	--	1,74
X	1,73	1,76

*Translator's note: commas represent decimal points.

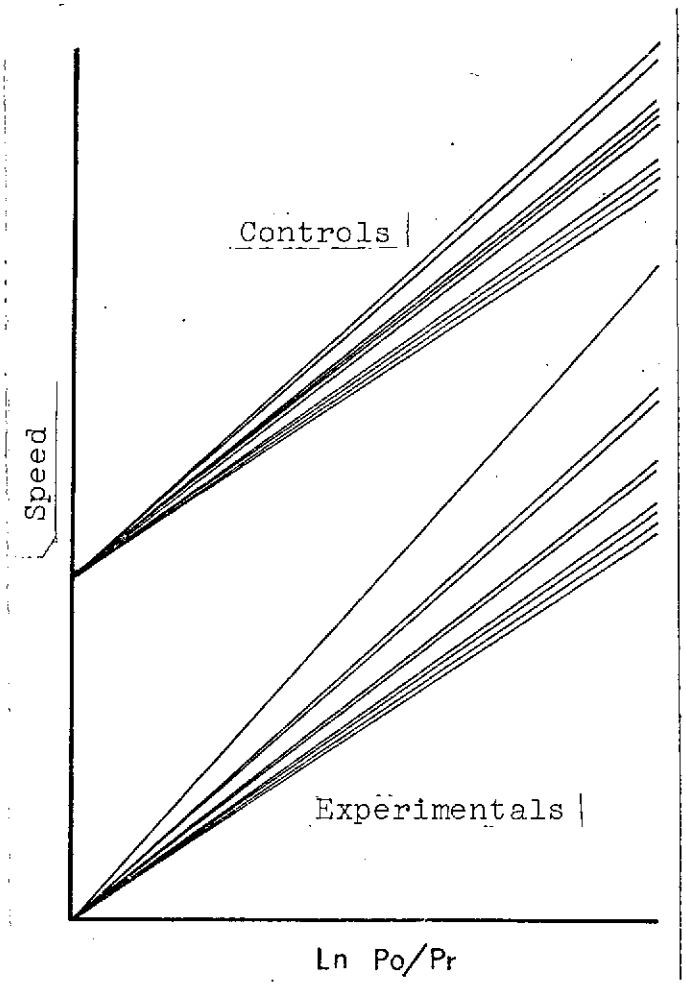

Figure 13. Lines of regression of the speed constant for the experimental solei and the control ones

Coefficient b (speed constant) varies little from one muscle to another. The average value in the control group is 1.76 cm per sec, with a standard error of the mean of 0.07. The average value for the experimental group of 1.79 cm per sec, with a standard error of the mean of 0.11.

The analysis of variance for the speed constant between the control groups and the experimental groups does not show any significant difference (Table 17).

Discussion

Our results show the existence of atrophy following immobilization with plaster during a period of 24 days. /337

TABLE 17. ANALYSIS OF VARIANCE OF SPEED CONSTANTS OF THE RIGHT SOLEI OF THE EXPERIMENTAL RATS AND OF THE CONTROL RATS*

Source of	Sum of squares	DL	Mean square	F.
Between groups	0,01	1	0,01	0,1429
Error	1,12	17	0,07	
Totals	1,13	18		—

* Translator's note: Commas represent decimal points.

Experimental animals, although consuming the same amount of food per gram of weight, exhibit a weight loss when compared to the control animals which, on the contrary, show a weight increase. While the experimental animals lose 6.17% of their initial weight, the controls gain 5.35%. This finding can be explained on the basis of a certain degree of dehydration of the experimental rats. In fact, control rats drink an average of 18% more water per day than the experimental rats.

The analysis of covariance which takes into account simultaneously the weight of the animals and that of the muscles shows a highly significant difference between the normal and the atrophied muscles.

Considering only the solei, we encountered an atrophy of 38.69% from the morphometric point of view, 33.57% with respect to the level of proteins, and 46.65% in the evaluation of tensions from the physiological point of view.

There is a great deal of variation between the experimental and the control group as to the tension developed. The value varies from an average of 164.6 g for the control group to an average of 86.6% for the experimental group. However, the intrinsic force of the

soleus remains the same [17], i.e., 2.6 kg per cm^2. Other investigators have found tensions of the order of 1.9 kg per cm^2 [24] up to 3.3 kg per cm^2 [37].

Here, the problem is connected with the dissection technique and the technique for the measurement of the muscle length in situ. The total weight of the soleus varies depending on the dissecting technique. Furthermore, the technique used for the measurement of the soleus length in situ allows differences of between 2 and 3 mm over a total length of about 35 mm.

The speed constant, although it has a high average value for the control group [4, 37], does not change following a prolonged period of immobilization. On the basis of these results, which are not significant but are rather interesting, one may assume that the muscle immobilized during a period of 24 days undergoes a loss of its basic elements which causes a decrease of its strength without modifying at all its reaction qualities.

Summary of Part II

The influence of immobilization upon the extensors of rats' feet has been investigated by studying the 40 solei, plantaris and gemelli of the hind feet of the animals.

The statistical analysis of the results shows significant differences with respect to the first three parameters studied, knowing the volume of the muscle, its content in proteins and the tension following stimulation with the pneumatic ergometer. The speed of contraction of the extensors remains constant in the two groups studied.

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